Folate Intake, Serum Homocysteine and Methylenetetrahydrofolate Reductase (MTHFR) C677T Genotype Are Not Associated with Oral Cancer Risk in Puerto Rico

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ABSTRACT We examined the relationships between folate and methionine intake, serum homocysteine levels (as a biomarker for folate metabolism), and methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism genotype and risk of oral cancer in a population-based, case-control study in Puerto Rico. Structured questionnaires were used to collect information on demographic factors, usual adult diet, and tobacco and alcohol use. Oral epithelial cells and blood samples were collected from a subset of subjects. Analyses were conducted by logistic regression, adjusting for age, sex, lifetime smoking and lifetime alcohol intake, with the following numbers of cases/controls, respectively: dietary data (341/521); MTHFR genotype (148/149); and homocysteine (60/90). Although increased folate intake was associated with decreased oral cancer risk [adjusted odds ratio (OR) in highest vs. lowest quartile = 0.6, 95% confidence interval (CI): 0.4, 1.0, $P_{trend} = 0.05$)], this finding was due almost entirely to folate intake from fruit (adjusted OR = 0.4, 95% CI: 0.2, 0.6; $P_{trend} = 0.0001$), whereas other dietary folate sources showed no clear association. Methionine intake and serum homocysteine levels were not associated with oral cancer risk. Subjects with the MTHFR C677T homozygous variant (TT) genotype had a nonsignificantly lower risk, and risk patterns tended to differ by level of folate, methionine, alcohol intake and smoking, although the power to detect significant associations in subgroups of these variables was low. Risks for oral cancer are not folate specific; preventive recommendations for this disease should emphasize the importance of a healthy diet, including substantial intake of fruits. J. Nutr. 132: 762–767, 2002.

KEY WORDS: • *folate* • *homocysteine* • *methylenetetrahydrofolate reductase* • *oral cancer* • *epidemiology*

The incidence of oral cancer (cancer of the oral cavity and pharynx) is higher in Puerto Rico (18.5 per 100,000 men; 4.5 per 100,000 women) (1) than among Hispanics on the U.S. mainland (8.9 per 100,000 men; 2.7 per 100,000 women) (2). We recently determined that the attributable risk of oral cancer due to alcohol and tobacco use in Puerto Rico was 76% for men and 52% for women (3), consistent with results from other localities (4–6).

In addition to these long-recognized risk factors for oral cancer, dietary patterns may also influence risk for this disease (7). In particular, several studies have indicated a protective role for fruits and vegetables (8–18), although the specific agents involved are uncertain. Folates, which have long been hypothesized to be related to cancer risk (19,20), are found in a wide variety of foods, particularly green leafy vegetables, grain products and orange juice (21). Metabolic reactions that require folate are called "one carbon-metabolism" reactions, and include amino acid metabolism, purine and pyrimidine synthesis, and formation of *S*-adenosylmethionine (SAM),[2] the agent primarily responsible for methylation of DNA (22). Disruption of one-carbon metabolism interferes with DNA synthesis, repair, and methylation, and thus may promote carcinogenesis (20). Importantly, the major known risk factors for oral cancer, smoking and alcohol use, can negatively influence folate metabolism, suggesting a mechanistic relationship among these factors and cancer risk (23–26).

To examine a potential link between factors in one-carbon metabolism and oral cancer risk, we evaluated folate intake, serum homocysteine (a sensitive indicator of folate status) (27) and methionine intake (methionine is converted to homocysteine in the one-carbon metabolism pathway), among participants in a population-based, case-control study of oral cancer in Puerto Rico. We also assayed for the C677T single

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[2] Abbreviations used: CI, confidence interval; ICD, International Classification of Diseases; MTHFR, methylenetetrahydrofolate reductase; OR, odds ratio; PCR, polymerase chain reaction; SAM, *S*-adenosylmethionine.

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nucleotide polymorphism in the gene encoding methylenetetrahydrofolate reductase (MTHFR), which is necessary for folate metabolism. The T allele reduces MTHFR enzyme activity and elevates plasma homocysteine levels (28); it has been associated with a decreased risk of colorectal cancer (29–31) and acute lymphocytic leukemia (32), and an increased risk of endometrial (33) and esophageal (34) cancers.

SUBJECTS AND METHODS

Through the Puerto Rico Central Cancer Registry and island pathology laboratories, we ascertained 519 newly incident, histologically confirmed, cases of cancer of the oral cavity (excluding lip and major salivary glands) and pharynx (excluding nasopharynx) [International Classification of Diseases (ICD)-9 codes 141, 143–146, 148, 149] (35) diagnosed between December 1, 1992 and February 28, 1995, in Puerto Rican residents aged 21–79 y. Beginning in February 1993, cancers of the major salivary glands (ICD-9 code 142) (35) were also included. Additional details of the study methods have been previously described (3,36).

Population-based control subjects with no history of oral cancer (n = 629) were selected from residents of Puerto Rico by two methods. To achieve an age-sex distribution similar to that of the case subjects, control subjects were selected by using probabilities based on the age-sex profile of oral cancer patients (Puerto Rico Central Cancer Registry, 1989–1990). For subjects <65 y old (n = 346), male-designated and female-designated households were selected from dwelling unit enumeration, within a two-stage area probability sample. Residents of the selected dwelling units were screened and study controls selected using age- and gender-specific sampling rates to approximate a 1:1 ratio with the cases. Controls aged ≥65 y (n = 283) were selected from the rosters of the Health Care Financing Administration (HCFA, Baltimore, MD) by systematic sampling, after a random start, to approximate the distribution of cases with regard to age (65–69 y, 70–74 y, 75–79 y) and gender. Three separate samplings from HCFA rosters were made during the course of field work, with approximately one third of the required controls identified during each sampling.

After exclusion of cases with cancer of the salivary gland, 341 cases (70% of eligible) and 521 controls (83% of eligible) were interviewed. Reasons for nonparticipation included death or illness (18% of cases, 2% of controls), subject refusal (5%, 8%), and difficulty in locating subjects or subjects had moved (6%, 7%).

At the time of the interview, subjects who lived in the San Juan metropolitan area were asked to provide oral epithelial cell specimens (buccal cells) and blood samples. The subjects who were agreeable later went to the University of Puerto Rico clinic, where a medical technician drew blood and obtained urine and buccal cell specimens. To increase the number of subjects from whom buccal cells were collected, part way through the study, subjects living outside the San Juan area were also asked to provide a buccal cell sample. These samples were collected in the home during the interview. Based on their residence and date of interview, 299 cases and 258 controls were eligible to provide buccal cells for genetic studies. These included all subjects in the San Juan metropolitan area (n = 150 cases/185 controls) as well as subjects outside the San Juan area who were interviewed after 6/28/94 (149 cases/73 controls). A total of 157 cases and 149 controls (53 and 58% of those eligible, respectively) provided buccal cell samples. Reasons for nonparticipation included death or illness (26% of cases, 4% of controls), subject refusal (8%, 24%), difficulty in locating subjects (8%, 14%), physician refusal (2% of cases), and other reasons (3%, 0.4%). Of the 157 cases, 9 subjects with cancer of the salivary gland were excluded from analyses; 13 cases and 3 controls were unable to be typed/missing, leaving 135 cases and 146 controls.

Blood samples were collected only at the clinic at the University of Puerto Rico in San Juan; therefore, only interviewed subjects who resided in the San Juan area (n = 150 cases, 185 controls) were eligible to donate blood samples used for the homocysteine analyses. A total of 66 cases and 91 controls (44 and 49% of those eligible, respectively) provided a blood sample. Reasons for nonparticipation included death or illness (31% of cases, 3% of controls), subject refusal (13%, 35%), difficulty in locating subjects (8%, 12%), physician refusal (0.7% of cases), and other reasons (3%, 0.5%). Excluded from the analyses were four cases with cancer of the salivary gland, one case with an extreme homocysteine concentration (186.12 μmol/L), which was suspected to be due to measurement error, one case with missing information and one control with a missing homocysteine measurement, leaving 60 cases and 90 controls.

Buccal cells were collected by brushing the selected mucosal surfaces with a small cytobrush, followed by a rinse with sterile water (36). Blood samples were allowed to clot at room temperature for a maximum of 6 h before being centrifuged, and serum samples were stored at −70°C. All subjects gave written informed consent to participate in the study. The study protocol was approved by the institutional review boards at the National Cancer Institute and the University of Puerto Rico.

Interview data

Trained interviewers used a structured questionnaire to collect detailed information on demographic factors, usual adult diet, and tobacco and alcohol use 1 y before the interview. Diet was assessed through 104 food and beverage frequency questions asking the subject to estimate usual consumption "during most of [their] adult life before one year ago." The food-frequency questionnaire was designed specifically for this study population. One subject (a male case) was excluded due to nonresponse to the entire diet section of the questionnaire. For 134 subjects with incomplete dietary data (11 subjects missing 3–5 food items, and 123 subjects missing 1–2 food items), missing answers were imputed from the median consumption by controls of the same gender. Nutrient indices were computed by multiplying each subject's food-frequency responses by a matrix of standard portion size and nutrient density estimates derived from USDA food composition tables (37) for 375 food items, including several USDA food items specific for foods consumed in Puerto Rico. Lifetime smoking was estimated from usual daily number of cigarettes smoked and total years of use. Lifetime alcohol intake was estimated from weekly intake, combining weekday and weekend amounts, and total years of use. One drink was considered equivalent to 42 g liquor (1.5 oz), 118 g of wine (4 oz) or 356 g (12 oz) of beer (3).

Laboratory analyses

Homocysteine. Serum homocysteine analyses were conducted using a modification of a HPLC method by Araki and Sako (38). An equivalent proportion of cases and controls was assayed in each of two batches. Each batch also included eight quality control samples (4 low and 4 high homocysteine level aliquots, masked as to level), which were monitored on the basis of rules of Westgard et al. (39). The CV was 4.9%, based on repeated measurements of the quality control material using the variance component estimation procedure in SAS (40).

MTHFR. A common single nucleotide polymorphism in MTHFR has a cytosine (C) to thymine (T) substitution at nucleotide 677, which results in an amino acid change from alanine to valine (28). The region surrounding position 677 in the MTHFR gene (National Center for Biotechnology Information Locus ID: 4524) was amplified using the polymerase chain reaction (PCR) technique, and the alleles were identified through restriction enzyme digestion (41).

PCR reactions of 15 μL contained 30 ng template genomic DNA and 1.5 U AmpliTaqGold DNA polymerase (Applied Biosystems, Foster City, CA) and the following concentrations of reagents: 0.16 μmol/L of each oligonucleotide primer, 1.6 mol/L of each dNTP, 15 mmol/L Tris-HCl (pH 8.0), 50 mmol/L KCl and 1.5 mmol/L $MgCl_2$. Reaction conditions were denaturation for 9 min at 95°C followed by 49 cycles at 94°C for 30 s and 62°C for 60 s, with a final extension step at 62°C for 10 min. Verification of amplification was obtained by comparing the 173-bp products with molecular weight standards in a gel made from a combination of 3% GibcoBRL Ultrapure agarose (Life Technologies, Grand Island, NY) and 1.5% NuSieve GTG agarose (BioWhittaker Molecular Applications, Rockland, ME) run for 1 h at 150 V and visualized with ethidium bromide. Reaction products (5 μL) were digested for 1 h at 37°C with 2.5 U of *Hinf I*

(New England Biolabs, Beverly, MA) in buffer at pH 7.9 of 10 mmol/L Tris-HCl, 10 mmol/L $MgCl_2$, 50 mmol/L NaCl and 1 mmol/L dithiothreitol. Genotypes were determined by analysis of restriction patterns after electrophoresis on agarose gels (as above) with T at position 677 resulting in fragments of 125 and 48 bp in length and with C at position 677 resulting in a single fragment of 173 bp. We refer to the CC genotype as wildtype, the CT genotype as heterozygous variant, and the TT genotype as homozygous variant.

Statistical analyses

Statistical analyses were conducted using SAS Version 6.12 and Version 8 for Windows (40). Correlations were calculated using the Spearman rank order correlation coefficient. Geometric means were calculated by transforming values with the natural logarithm, calculating the mean and then transforming back to standard units.

The odds ratio (OR) was the measure of association used to estimate the relative risk of oral cancer. Folate, methionine, and homocysteine quartiles were based on the frequency distribution among the controls. Unconditional logistic regression was used to obtain maximum likelihood estimates of the OR and a 95% confidence interval (CI), while adjusting for potential confounders (42). Control for confounding was considered adequate when the addition of a potential confounder or an increase in the number of strata of a confounder did not change the adjusted OR by ≥10%. Most OR in the text and tables are adjusted for age (≤65, 66–71, >71 y), sex, lifetime smoking (nonsmoker, ≤10,000 packs, >10,000 packs, smoker of tobacco other than cigarettes), and lifetime alcohol intake [nondrinker, ≤9647, 9648–42,000, >42,000 drinks (cut-off points are based on tertiles among the controls)]. Because the buccal cell collection differed by place of residence, the MTHFR OR were additionally adjusted for this factor. For models of folate and methionine intake, which had a larger number of subjects, the OR were adjusted with more categories for the confounder variables (age: ≤55, 55–59, 60–64, 65–69, 70–74, >75 y; lifetime smoking: nonsmoker, ≤5,000, 5,001–10,000, 10,001–20,000, >20,000 packs, smoker of tobacco other than cigarettes; lifetime alcohol intake: nondrinker, ≤10,000, 10,001–40,000, 40,001–80,000, >80,000 drinks) to be consistent with our previous publications. This did not materially change the OR compared with models with fewer categories for the confounder variables. The addition of education (≤12 y/high school, >12 y) and income (<$10,000, $10,000–$14,999, $15,000–$19,999, ≥$20,000, unknown) did not materially affect the OR; thus, they were not included in the models. To explore subgroup effects, the OR for MTHFR genotype were calculated within strata of folate and methionine intake and lifetime tobacco and alcohol use. Tests for trend were obtained by assigning the score 1–4 to each quartile and treating this categorical variable as continuous. All statistical tests were two-tailed. $P < 0.05$ or a CI that excluded 1.0 was considered significant.

RESULTS

Cases and controls were similar with respect to sex and place of residence. Cases were significantly older (mean age 63.2 vs. 61.0. $P = 0.003$) and reported lower incomes and less education. Controls reported less use of tobacco and alcohol. Further details have been published (3,36).

When analyzed as total intake from foods, folate was inversely associated with oral cancer risk (OR = 0.6, 95% CI: 0.4, 1.0 for highest vs. lowest quartile, **Table 1**). However, with adjustment for fruit intake, the association disappeared (OR = 1.1, 95% CI: 0.6, 2.2 for highest vs. lowest quartile, Table 1). We further examined folate intake among nonusers and heavy users of alcohol and tobacco and found no systematic pattern of risk. Methionine intake was not significantly associated with risk (OR = 1.4, 95% CI: 0.9, 2.2 for highest vs. lowest quartile, Table 1).

When folate intakes from dairy products, fruits, grains, legumes, meats and vegetables (excluding legumes) were examined separately, only folate derived from fruits showed an inverse association with oral cancer risk (OR = 0.4, 95% CI: 0.2, 0.6 for highest vs. lowest quartile, **Table 2**). Fruit was the highest contributor to folate intake, with a median folate intake from fruit of 110.1 μg/d. Median folate intakes from the other food groups were as follows: vegetables, 90.72 μg/d; legumes, 60.78 μg/d; dairy, 37.04 μg/d; grains, 31.15 μg/d; and meat, 13.75 μg/d (see Table 2 for quartile distributions). The correlation between folate intake and fruit intake was 0.6 among the controls ($P < 0.0001$).

The correlation between folate intake and vitamin C intake was 0.8 ($P < 0.0001$) among the controls. However, the OR for folate derived from fruit, adjusted for vitamin C derived from fruit, was 0.5 for the highest vs. lowest quartile (95% CI: 0.2, 0.9), whereas the OR in this model for vitamin C derived from fruit was 0.9 for the highest vs. lowest quartile (95% CI: 0.4, 1.7). These data indicate that the inverse association with folate derived from fruit is not due to vitamin C.

Among controls, homocysteine levels were not associated with folate intake ($r = -0.1$, $P = 0.3$). The geometric mean homocysteine level tended to be higher in cases, but the difference was not significant (10.6 μmol/L among cases, 9.7 μmol/L among controls, $P = 0.1$). The unadjusted OR for oral cancer were nonsignificantly elevated with increased homocysteine levels [OR for lowest to highest quartiles were, respectively: 1.0, 1.1, 1.4, 1.8 (95% CI: 0.7, 4.7)]. However, once adjustment was made for age, sex, lifetime smoking and lifetime alcohol intake, the risk was attenuated [OR for lowest to highest quartiles were, respectively: 1.0, 0.8, 1.0, 1.0 (95% CI: 0.3, 3.4)].

The MTHFR homozygous variant (TT) genotype was weakly associated with a decreased oral cancer risk (adjusted OR = 0.5, 95% CI: 0.2, 1.4, **Table 3**). This was true whether the individuals with the homozygous variant genotype were compared with those with the wildtype genotype only, or with those with the heterozygous variant and the wildtype genotypes combined. Geometric mean homocysteine levels did not differ by MTHFR status for either cases or controls.

We further examined MTHFR genotype stratified by folate

TABLE 1

Oral cancer risk by folate and methionine intake for women and men in Puerto Rico

Range of intake	Cases (n = 341)	Controls (n = 521)	OR[1] (95% CI)	OR[2] (95% CI)
	n			
Folate, *μg/d*				
<316.2	113	130	1.0	1.0
316.2–399.7	79	130	0.8 (0.5, 1.2)	1.0 (0.6, 1.6)
399.8–518.6	86	131	0.8 (0.5, 1.3)	1.2 (0.7, 2.2)
≥518.7	63	130	0.6 (0.4, 1.0)	1.1 (0.6, 2.2)
			$P_{trend} = 0.05$	$P_{trend} = 0.56$
Methionine, *g/d*				
<1.65	77	130	1.0	N/A[3]
1.65–2.08	75	130	0.9 (0.6, 1.5)	
2.09–2.54	77	131	0.8 (0.5, 1.4)	
≥2.55	112	130	1.4 (0.9, 2.2)	
			$P_{trend} = 0.21$	

[1] Adjusted for age, sex, lifetime smoking and lifetime alcohol intake; OR, odds ratio; CI, confidence interval.

[2] Adjusted for age, sex, lifetime smoking, lifetime alcohol intake and fruit intake.

[3] Methionine analyses are not adjusted for fruit intake because this is not applicable.

TABLE 2

Oral cancer risk by folate intake from dairy, fruits, grains, legumes, meats, and vegetables for women and men in Puerto Rico

Range of intake	Cases (n = 341)	Controls (n = 521)	OR[1] (95% CI)
	n		
Folate from dairy products, $\mu g/d$			
<19.41	86	130	1.0
19.41–37.03	80	130	1.3 (0.8, 2.1)
37.04–53.99	96	131	1.5 (0.9, 2.5)
≥53.99	79	130	1.2 (0.7, 1.9)
			P_{trend} = 0.41
Folate from fruits, $\mu g/d$			
<75.99	133	130	1.0
75.99–110.09	73	130	0.5 (0.3, 0.7)
110.10–155.9	81	131	0.5 (0.3, 0.7)
≥155.9	54	130	0.4 (0.2, 0.6)
			P_{trend} = 0.0001
Folate from grains, $\mu g/d$			
<19.32	93	130	1.0
19.32–31.14	91	130	0.8 (0.5, 1.3)
31.15–60.40	92	131	0.9 (0.5, 1.4)
≥60.40	65	130	0.9 (0.5, 1.4)
			P_{trend} = 0.62
Folate from legumes, $\mu g/d$			
<39.79	47	130	1.0
39.79–60.77	105	130	1.8 (1.1, 3.0)
60.78–74.48	93	131	1.7 (1.0, 2.9)
≥74.48	96	130	1.6 (0.9, 2.7)
			P_{trend} = 0.18
Folate from meats, $\mu g/d$			
<7.72	46	130	1.0
7.72–13.74	97	130	1.3 (0.7, 2.1)
13.75–21.23	92	131	1.2 (0.7, 2.0)
≥21.23	106	130	1.1 (0.6, 1.8)
			P_{trend} = 0.99
Folate from vegetables, $\mu g/d$			
<66.95	107	130	1.0
66.95–90.71	102	130	1.1 (0.7, 1.7)
90.72–126.80	60	131	0.7 (0.4, 1.1)
≥126.8	72	130	1.0 (0.6, 1.6)
			P_{trend} = 0.50

[1] Adjusted for age, sex, lifetime smoking, and lifetime alcohol intake; OR, odds ratio; CI, confidence interval.

TABLE 3

Oral cancer risk by methylenetetrahydrofolate (MTHFR) C677T genotype for women and men in Puerto Rico

MTHFR genotype	Cases (n = 135)	Controls (n = 146)	Adjusted OR[1] (95% CI)
	n		
CC	67	69	1.0
CT	53	62	0.6 (0.3, 1.2)
TT	15	15	0.5 (0.2, 1.4)
TT vs. CC and CT combined			0.6 (0.2, 1.6)

[1] Adjusted for age, place of residence, sex, lifetime smoking, and lifetime alcohol intake; OR, odds ratio; CI, confidence interval.

intake, methionine intake, lifetime smoking and lifetime alcohol intake. Subjects with the homozygous variant genotype (TT) who fell into the "low risk" group (high consumption of folate or methionine or low levels of lifetime smoking or alcohol intake) tended to have a lower risk of oral cancer than those with the CC and CT genotypes (**Table 4**), whereas those in the "high risk" group were not protected by the TT genotype. However, the numbers of subjects in the subgroups were small and none of the OR were significant. Although numbers were even smaller, the association with serum homocysteine followed a similar pattern. Subjects with the TT genotype and the "low risk" characteristic of low homocysteine levels had an OR = 0.3 (95% CI: 0.02, 3.6).

DISCUSSION

In our population-based, case-control study in Puerto Rico, high folate intake was associated with a significantly reduced risk of oral cancer; however, this seemed to be due solely to fruit intake. We found no associations with folates from other food sources (Table 2), indicating that a causal association

TABLE 4

Adjusted odds ratios (95% confidence interval) of oral cancer risk by methylenetetrahydrofolate (MTHFR) C677T genotype stratified by folate intake, methionine intake, lifetime smoking and lifetime alcohol intake for men and women in Puerto Rico[1]

MTHFR genotype	High risk group[2]	Low risk group
	Folate intake	
	<332.7 μg/d	≥332.7 μg/d
CC and CT	1.0[3]	1.0[3]
	54/43[4]	66/88
TT	1.20 (0.2, 6.0)	0.4 (0.1, 1.3)
	9/4	6/11
	Methionine intake	
	<1.78 g/d	≥1.78 g/d
CC and CT	1.0[3]	1.0[3]
	36/41	84/90
TT	0.9 (0.2, 4.7)	0.3 (0.1, 1.1)
	10/5	5/10
	Lifetime smoking	
	>5,000 packs	≤5,000 packs
CC and CT	1.0[3]	1.0[3]
	96/47	24/84
TT	1.1 (0.3, 3.8)	0.6 (0.1, 4.4)
	13/6	2/9
	Lifetime alcohol intake	
	<40,000 drinks	≤40,000 drinks
CC and CT	1.0[3]	1.0[3]
	85/39	35/92
TT	0.8 (0.2, 2.9)	0.4 (0.1, 2.0)
	13/5	2/10

[1] Adjusted for age, place of residence, sex, lifetime smoking (except the smoking analysis) and lifetime alcohol intake (except alcohol intake analysis).

[2] Groups are separated at low folate or methionine tertile and the point at which risks start to increase for lifetime smoking and lifetime alcohol intake.

[3] Referent group.

[4] Cases/controls.

with folate is unlikely. We found no clear pattern of risk for oral cancer with methionine intake or serum homocysteine levels.

Numerous studies have shown a reduced risk of oral cancer with increased fruit and/or vegetable consumption (8–18), although the evidence is not entirely consistent (43). Few studies, however, have examined the role of folate specifically. In one study, serum folate levels were significantly decreased in subjects with oral leukoplakias (44), whereas in another study, folate intake was negatively associated with risk of oral and pharyngeal cancer (45). Folate was not consistently related to risk in two other studies (9,46). Because the observed protective effect of folate in our study was restricted to folate from fruit, the evidence suggests that fruit itself, or some component of fruit, may be the protective factor.

Disruption of one-carbon metabolism interferes with DNA synthesis, repair and methylation, and thus may promote carcinogenesis (20). Efficient one-carbon metabolism requires adequate levels of folate, as well as several other vitamins, and optimal activity of several enzymes. In one-carbon metabolism, methionine is activated by ATP to form SAM, a methyl donor to DNA, and the by-product of this reaction is hydrolyzed to homocysteine (47,48). There are two metabolic pathways for removal of homocysteine. It can be degraded in a reaction requiring vitamin B-6 or it can be converted back to methionine in a reaction requiring vitamin B-12, by accepting a one-carbon group from folate (47,48). The MTHFR enzyme provides folate in the proper form for this latter step (22). Impairment of either pathway by, for example, low folate, B-6 or B-12, or reduced MTHFR activity, can result in the accumulation of homocysteine (28,48).

Elevated serum homocysteine levels have been related to colorectal cancer risk as a main effect (49) and in conjunction with the MTHFR C677T homozygous variant genotype (TT) (50). Elevated homocysteine has also been related to risk of cervical cancer (51,52) and cervical dysplasia (53), although not consistently (54). Elevated homocysteine has been inversely (but nonsignificantly) related to pancreatic cancer risk (55). In the current study, however, we found little evidence for an association between serum homocysteine levels and oral cancer risk. Blood samples were taken after disease onset; therefore, disease could have caused an elevation in homocysteine levels among the cases. However, this type of bias would have resulted in an inflated risk for homocysteine, and we found no evidence of risk. Homocysteine is a sensitive indicator of folate inadequacy (27), and the lack of an association between homocysteine levels and oral cancer supports the lack of a direct relationship between folate and oral cancer, although the numbers are small.

Subjects with the MTHFR homozygous variant (TT) genotype tended to have reduced risk of oral cancer, although this relationship was not significant. Further, although based on a small number of subjects, those with the homozygous variant genotype and "low risk" characteristics of high consumption of folate or methionine, or low lifetime smoking or alcohol intake tended to have decreased risk of oral cancer. This evidence of potential effect modification will have to be evaluated in larger studies. Interestingly, studies of colorectal adenomas and cancer, with a few exceptions, have found similar effect modification relationships, especially with folate (29–31,50,56–60).

The use of a population-based sampling design was one of the strengths of this study; however, self-selection or survival-related biases, especially for the buccal cell and serum components of the study, may have influenced our findings. Only 53 and 58% of eligible cases and controls, respectively, donated buccal cells used for the MTHFR analysis, and only 44 and 49% of eligible cases and controls, respectively, donated blood used for the homocysteine analysis. We compared those who donated buccal cells and blood with all who were interviewed on age, sex, race, place of residence, education and income, and observed some differences in education and income. However, controlling for education and income did not materially affect the OR. In addition, mean folate intakes were similar ($P = 0.9$) in the group that was interviewed (396.65 μg/d) and in the subsample that donated biospecimens (395.48 μg/d), indicating that any participation bias in the folate-related analyses would be minimal.

Our major finding is that increased folate intake, although associated with oral cancer, does not appear to be causally linked to this disease. When folate from specific food groups was examined, only folate from fruits was associated with a decreased risk of oral cancer. In addition, serum homocysteine levels, a biomarker for folate status, were not associated with risk. Preventive measures for oral cancer should emphasize the importance of a healthy diet, including substantial intakes of fruits.

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